2471-Pos Board B163
Promoter Architecture Dictates Variability in Gene Expression
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Recent experimental studies have reported that cell-to-cell variability in gene expression is a universal function of mean gene expression levels, implying that knowledge of mean levels of gene expression alone is sufficient to determine the level of variability. This result seems at odds with the physical intuition underlying efforts in the literature to predict the level of variability for different promoter architectures (where we take "promoter architecture" to mean the numbers, positions, and strengths of transcription factor and RNA polymerase binding sites). For instance, we would expect slow TF binding/unbinding kinetics to yield a relatively higher level of variability at a given mean than fast kinetics, as the switching between distinct transcriptional states will yield a broad distribution in mRNA production.
To address this apparent conundrum, we constructed a set of 18 constitutive (unregulated) promoters with expression levels spanning a range of 2.5 orders of magnitude, and found that the observed mRNA distributions were consistent with Poissonian production of mRNA. We then placed one of these promoters under regulation by the repressor transcription factor LacI, and measured the resulting mRNA distributions at a range of LacI concentrations. We found that, for the regulated promoter, the level of variability as a function of mean expression followed a distinct, promoter architecture dependent curve as compared with the unregulated promoter. These results demonstrate that the level of variability in gene expression depends on the specific promoter architecture in play.

2472-Pos Board B164
Single Molecule Investigation of RNA Polymerase I using Multiplexed Tethered Particle Motion
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In eukaryotic cells, RNA Polymerase I (Pol I), which produces ribosomal RNA (rRNA), catalyzes up to 80% of all transcriptional activity in growing cells, and its regulation is an emerging field of study for anti-cancer drug development. In order to gather more comprehensive information about ribosomal RNA production by Pol I, we conducted single molecule experiments to characterize rates and pauses in the elongation phase of transcription by wild-type and mutant Pol I. Transcription by individual molecules was based on real-time measurements using custom multiplexed Tethered Particle Motion (TPM) microscopy. Microspheres were attached to one end of a DNA template engaged in an elongation complex with a Pol I molecule immmuno-imobilized on a coverslip. The range of Brownian motion of the microsphere was a function of the tether length that in turn depended on the progress of the polymerase. Either the lack of a full complement of associated factors or the constitution of in vitro transcription assay limited of polymerase activity to only 1-2%. In order to increase the throughput of the transcription assay, we increased the field of view and implemented a particle-tracking algorithm to track a high number of beads simultaneously in real time. The time courses of rare transcription events were analyzed for velocity and pausing. The processivity of Pol I without tension on the DNA template was remarkably lower than observed in studies of Pol II transcribing DNA templates under a few picoNewtons of tension. Furthermore, as reported previously based on bulk experiments, the average elongation rate of an F1205H mutant was lower than that of wild-type Pol I. At least six possible pause sites were also identified including one recently found in bulk transcription study.

2473-Pos Board B165
The Transcription Factor Titration Effect Dictates Level of Gene Expression
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Models of transcription are often built around a picture of RNA polymerase and transcription factors acting on a single copy of a promoter. However, many transcription factors are shared between multiple genes with varying binding affinities. Beyond that, genes often exist at high copy number; in multiple, identical copies on the chromosome or on plasmids or viral vectors with copy numbers in the hundreds. Using a thermodynamic model, we characterize the interplay between transcription factor copy number and the demand for that transcription factor. Using video microscopy, we measure this effect and demonstrate the parameter-free predictive power of the thermodynamic model as a function of the copy number of the transcription factor and the number and affinities of the available specific binding sites. Understanding how to account for the effects of competing binding sites is an important facet of predictive control of transcription and gene circuit design, where transcription factors regularly navigate complex DNA binding landscapes.

2474-Pos Board B166
Inhibition of Tumor Cell Growth through the Environmental Epigenetic Regulation at 2ATA Elevated Pressure (EP) in H460 Lung Cancer Cells
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We have examined the elevated pressure (EP), an extrinsic mechanical force, applied to cells or tissues for diverse cellular biological properties such as cell growth, oxidative stress and DNA damage. We found that cell growth was inhibited without metabolic change or cellular damage, and sensitizing effect for TRAIL-induced apoptosis in various cancer cell lines. In vivo, a CAM model, we have found synergistic effect of EP on tumor suppression with the anticancer drug treatment and cell proliferation was decreased about 25% under EP. To understand the effect of the EP on tumor suppression related epigenetic status, we analyzed DNA methylation of tumor suppressor genes. DNA methylation regulates chromosomal stability and gene expression within the epigenetic mechanisms which is finely regulated during development, differentiation and mature stage of multicellular organisms. Epigenetic states are reversible and modified by environmental factors, which may contribute to the development of abnormal phenotypes. The relations between epigenetic states and environmental signals of human are less defined than plants and mice. However, it has been studied about the interrelationship between aging related hypo- and hypermethylation and diseases such as cancer. There are few studies about epigenetic regulation by extrinsic mechanical force such as elevated pressure. We analyzed DNA methylation of the selected target gene LOXL1, increased by 2fold at EP through comparative transcriptome analysis previously, as well as biomarker genes in lung cancer cells. We found that LOXL1 promoter methylation has been decreased by 30% and the gene expression in H460 lung cancer cells has been increased at elevated pressure. Our results suggest that EP, as the environmental factor, can induce epigenetic change and also inhibit tumor cell growth through the regulation of the DNA methylation.

Ribosome and Translation

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Critical Decoding Step in the Ribosome Revealed by Dynamical Network Analysis
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The ribosome needs to select the correct (cognate) aminoacyl-tRNA (aa-tRNA) among all the available aa-tRNAs to carry out protein synthesis. During the selection of the cognate aa-tRNA, also known as the decoding step, a ternary complex consisting of an aa-tRNA along with the elongation factor Tu (EF-Tu) and a GTP molecule approaches the ribosomal A-site. If the codon-anticodon pair matches in the decoding center, GTP hydrolysis occurs more than 75 Å away, which gives rise to conformational change in EF-Tu and its detachment from the ribosome. The molecular mechanism of the decoding process, which occurs very rapidly (about 20 amino acids per second), and very accurately, is unknown despite decades of intense study. According to experimental data, helices h8 and h14 in 16S rRNA play a crucial role in the activity and fidelity of the decoding process. These two helices together interact with two protein helices of the large subunit, L14 and L19, to form bridge 8 (B8), which connects the two ribosomal subunits. All-atom molecular dynamics simulations were performed on the complete ribosome for 150 ns. Using the Dynamical Network Analysis plugin in VMD, a network was assigned to the system based on cross-correlations of the residues' displacements along the trajectory. Inter-correlated communities in the network and the strongest pathways between the decoding center and the GTP binding site were calculated to shed light on how codon-anticodon pairings regulate the GTP hydrolysis. Finally the results for the wild-type ribosome were compared with the results for ribosomes carrying mutated/deleted/inserted base pairs in their h8 and h16 helices in order to reveal the functional importance of these helices in the decoding process at the molecular level.